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Betterment the production of prodigiosin that was produced from *Serratia marcescen* by means mutations.

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Abstract: *Serratia marcescens was exposed to mutation for becoming better the production of prodigiosin (quantitatively and qualitatively) and examined it as antibacterial activities. One isolate of S.marcescen (**Wild**) was exposed to different kinds of antibiotics (Gentamycin 10µg/ml) to produce mutant isolates from S.marcescen and examined to produce prodigiosin. The isolate that produce the largest amount of prodigiosin among four isolates was used for more studies. Staphyllococcus Baciilus, E.coli, and Pseudomonas were exposed to prodigiosin for testing inhibiting activity of it against these bacterial strains.*
Keywords: *Serratia marcescens, prodigiosin, Gentamycin, mutation*

I. Introduction

Prodigiosin (red pigment) is a member of prodigiosins produced by *Serratia*, actinomycetes and few other bacteria. Different concentrations of prodigiosin were studied against cancerous cells. It was involved in apoptosis of haematopoietic cancer cell [2]. Serratiae is a Gram negative bacteria, opportunistic pathogen for human, plant and insect and member of enterobacteriaceae. Strains of *S. marcescens* produce nosocomial mial infections that are clinically problematic because multidrug resistance. *S. marcescens* has been isolated from different niches (soil, water, plants and air), *S.marcescens* is a non-motile, citrate positive bacterium. On nutrient agar, the colonies are convex, circular with entire margin [2]. Some *S. marcescens* strains were able to grow in a wide range of ecological niches has been attached to the protection of a spectrum of extracellular products including bacteriocins, chatinase, proteases, nucleases, lipases, and surfactants, wetting agents [3]. A bifurcated pathway has been proposed for the productions of prodigiosin culminating in the enzymic condensation of the final products of the two pathways, 4-methoxy-2,2-bipyrrole-5-carboxyaldehyde (MBC) and the monopyrrole, 2-methyl-3-n-amyl-pyrrole (MAP). The precursors for prodigiosin were seemed to be acetate, serine, alanine, methionine and proline [4]. Recently, the mechanism of proline incorporation into a pyrrole moiety has been shown biochemically and a pathway for production of undecylprodigiosin proposed [3]. Prodigiosin have no special role in the physiology of strains, but have been reported to have antibacterial, antifungal and antiprotozoal/antimalarial activities [5]. They are of interest because they might have critical clinical utility. Prodigiosins and synthetic derivatives have been shown to have main and specific immunosuppressive activity, with novel targets clearly distinct from other drugs [6]. Many possible mechanisms are suggested attributed to prodigiosins as pH modulators, cell cycle inhibitors, DNA cleavage agents and mitogen activated protein kinase regulators [7]. These molecules when combined with some other anticancer agents can help in fighting cancer [8]. The mmunosuppressive characters were also carried earlier by Han et al. (1998) [9]. Effect of prodigiosin on human carcinoma cells was tested by Kamble, et al. (2012), and significant results were found. Spodoptera litura is classified as one of the crop destructing insect. Bacillus thuringiensis toxin viz Cry1C is the major weapon used against these insects, insecticidal activity was found to be increased when prodigiosin was combined with Cry1C [8]. For the reasons above, the importance of prodigiosin was revealed. Therefore, the amount of prodigiosin was hoped to be increased by doing spontaneous mutation. In present study the antibacterial effect of prodigiosin was evaluated.

II. Materials and Methods

2.1 Selected isolates Environmental isolates were collected, examined for growing of *Serratia*, many isolates were identified as *S.marcescens* that had the ability to produce the red pigment (prodigiosin) (**Fig** 1).

2.2 Extraction of prodigiosin

The isolates were examined for production of prodigiosin to select the best isolate in prodigiosin production. The best isolate that produce prodigiosin in large amount was called ***wild.***

2.3 Nutrient agar antibiotic medium for gradient method [10,12]

The nutrient agar was prepared as per the manufacturer's instructions (Himedia, India). The medium was mixed with antibiotic solution at 40 °C and poured in sterilized plates (fo gradient plates and selective plates) and incubated for 24 h to insure the sterility [10-12].

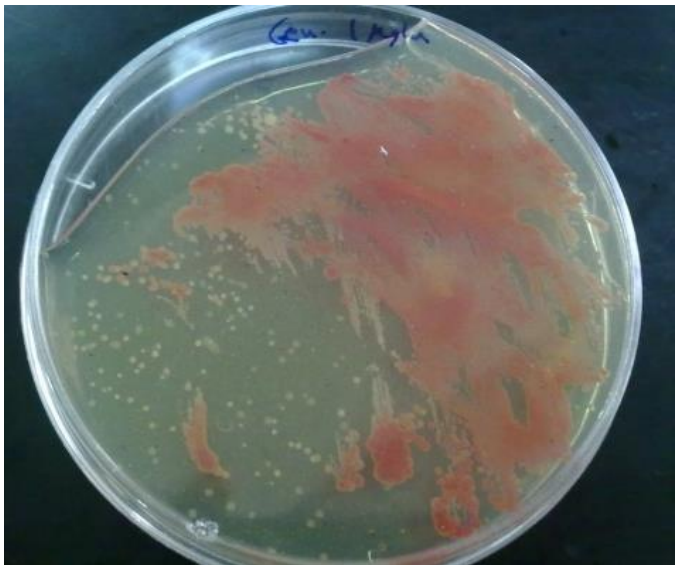

Fig 1. *S. marcescens* isolate *wild* produced prodigiosin attributed with red pigment.

This medium was used for isolation bacterial mutant of different antibiotics. Two hundred of overnight broth medium was transferred to nutrient agar gradient plates, spread by sterilized spreader carefully, Incubated at 37°C for 24 h. The growth of bacteria was examined according to action of antibiotic concentration on gradient plates (**Fig 2**).

2.4 Production of spontaneous mutant isolates

The selected isolate of *S. marcescens*, ***wild*** isolate was exposed to various concentrations of different types of antibiotics to produce mutated isolates. Two hundred of nutrient broth culture (overnight 18 h) was transferred to nutrient agar antibiotic plates for different antibiotic with different concentrations (according the estimation ofactive concentration by gradient method, spread by sterilized spreader, then incubated at 37°C for 24 h. The number of mutants appeared colonies were counted. Small part of selected colonies were streaked by sterilized loop on the same nutrient agar antibiotic medium , which the colonies were selected from it to be sure these colonies were mutant and not physiological adaptation compared with type strain , the colony which failed to grow was to be not real mutant.

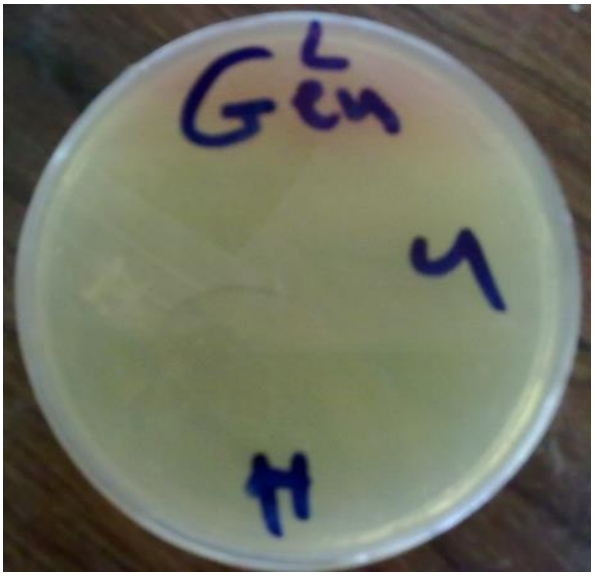

Fig 2. Gradient plate inoculated with *S. marcescens* and incubated for 37°C for 24 h.

2.5 Extraction of prodigiosin pigment

Three of mutant isolates G1-4, G1-2, G0.5-4, and the wild isolate were tested and the comparison among them was done according to the amount of prodigiosin pigment produced.

2.6 Antimicrobial activity of prodigiosin

Four isolates, *Pseudomonas*, Staphylococcus, Bacillus, *Escherisea coli* were tested for susceptibility against prodigiosin pigment.

2.7 Statistical analysis

Statistical software SPSS 10.0 was applied in this experiment. Significance between produced prodigiosin by selected isolate and other mutant isolates was estimated.

III. Results and discussion

Two isolates were identified as *S. marcescens*, one of the two isolates was been able to produce prodigiosin in perceptible amount called ***wild***, while another one was not been able to produce prodigiosin in perceptible amount, the second isolate was neglected, because it could not been able to produce prodigiosin and this disagrees with the aim of this study, (production prodigiosin pigment in large amounts). After purification of ***wild*** isolate, it was examined again for its ability to produce prodigiosin. After that it was exposed to solution of different kinds of antibiotics (gentamycin, ceftriaxone, cefalexin) with different concentrations, gentamycin was the only antibiotic was not decrease or prevent production of prodigiosin. In **fig 3** the growth that exposed to cefataxime showed no prodigiosin production, while the plate that exposed to gentamycin was not effected on the prodigiosin production.

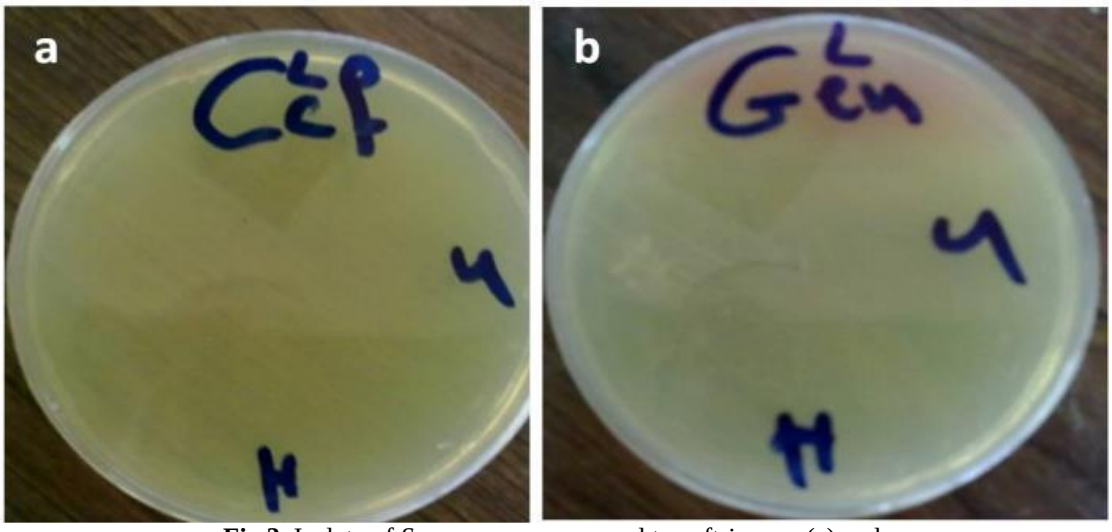

Fig 3. Isolate of *S. marcescens* exposed to ceftriaxone (a) and gentamycin (b).

Gentamycin antibiotic was mixed with nutrient agar media to produce spontaneous mutants from wild (**Fig 4**).

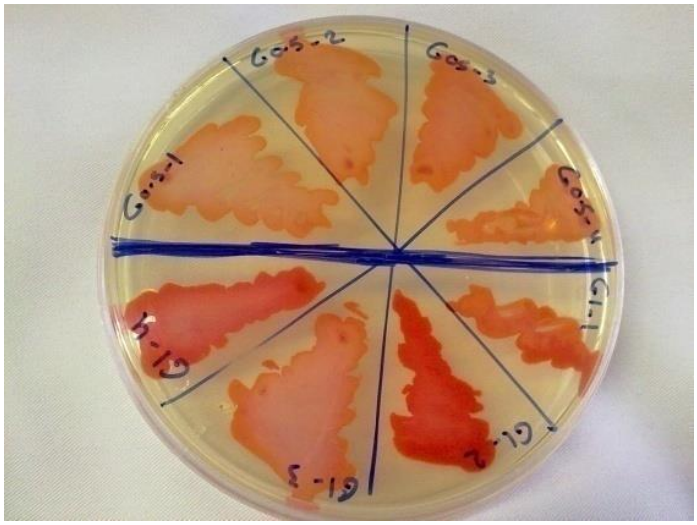

Fig 4. Different mutant isolates of *Serratia* grown on medium contains gentamycin.

Many mutant isolates were isolated from wild isolate (**Fig. 4**), and examined for prodigiosin production. Three mutant isolates shown in table 1. Production of prodigiosin in in mutant isolates was higher than wild isolate. The ***wild*** isolate succeeded to grow on the regions of low concentrations of antibiotics, while failed to grow on the regions of high concentrations. That means the bacteria; ***wild*** isolate could resist the low concentrations of antibiotics and survival again and again because mutations were happened for these bacteria make it resist the presence of antibiotics that must kill it. These isolates were called **spontaneous mutants**.

While, the death of isolates on media with antibiotics when still inoculated on the same media with antibiotics that's mean there is no real mutation and the bacteria adapt the low concentrations in the first time but could not still growing in the presence of this antibiotic. The results were showed that the amount of prodigiosin produced by ***wild*** isolate increased after spontaneous mutation (**Table 1**). The reason of production large amount of prodigiosin after obtaining mutant isolates, is the spontaneous mutant isolate was stronger than wild isolate and had more able to produce prodigiosin [12].

The activity of prodigiosin that produced in largest amount by mutant isolate had not changed their activity after applying spontaneous mutation, the production of prodigiosin from *S. marcescen* that produced in liquid media was more than amount produced by solid media, and this was agreed with previous study [13].

Table 1. Production of prodigiosin from *wild* and mutant isolates.

isolates	Gentamycin concentration (mg/ml)	Amount of produced prodigiosin (µg)
wild	Without	150000
G1-2	1	280000
G1-4	1	190000
G0.5-4	0.5	250000

There is significant difference ($P<0.05$) in production of prodigiosin by wild isolate and three mutant isolates, while there is no significant differences ($P<0.05$) in comparison with other mutant isolates. An application of produced prodigiosin by wild isolate and other three mutant isolates was examined. This application was antibacterial activity of prodigiosin produced by wild isolate and the three mutant isolates against different Gram positive and negative bacteria (**Table 2**). The red pigment was showed a serious role in inhibition the growth of bacteria, and this was agreed with earlier studies [13, 7]. The method of inhibition of prodigiosin was returned to considering prodigiosin as cell cycle inhibitors, and DNA cleavage agents [1]. The amount of prodigiosin was increased because the great role of prodigiosin as one of new experimented way to fight cancer, effect of prodigiosin on human carcinoma cells was investigated [14, 9]. Prodigiosin combined with other materials to enhance its activity, like combination between toxin viz Cry1C that was extracted from *B thuringiensis* and prodigiosin, this combination is the major weapon employed against *Spodoptera litura* that is one of the crop destructing insect, the insecticidal activity was found to be enhanced when prodigiosin was combined with Cry1C. [14-16]. the present study proved strongly the direct role of prodigiosin in inhibiting bacterial growth.

Table 2. Antibacterial activity of prodigiosin of *wild* isolate and three mutant isolates against *Pseudomonas, Staphyllococcus, Bacillus and E. coli*.

	Pseudomonas	***Staphyllococcus***	***Bacillus***	***E. coli***
Wild	9mm	10mm	9mm	7mm
G1-2	9mm	11mm	10mm	7mm
G1-4	9mm	10mm	8mm	8mm
G0.5-4	8mm	8mm	16mm	8mm

Control - - - -

The results in **table 2** showed that the antibacterial activity of prodigiosin produced from wild isolate and three mutant isolates against different Gram positive and negative bacteria, and revealed that prodigiosin pigment (whether extracted from wild isolate or from mutant isolates) had antibacterial activity against both Gram positive and negative bacteria. Prodigiosin pigment had inhibition activity against different bacteria isolates with concentration of 1000 µg/ml.

Fig. 5 showed significant effect (P<0.05) at the concentration of prodigiosin (1000 µg/ml).

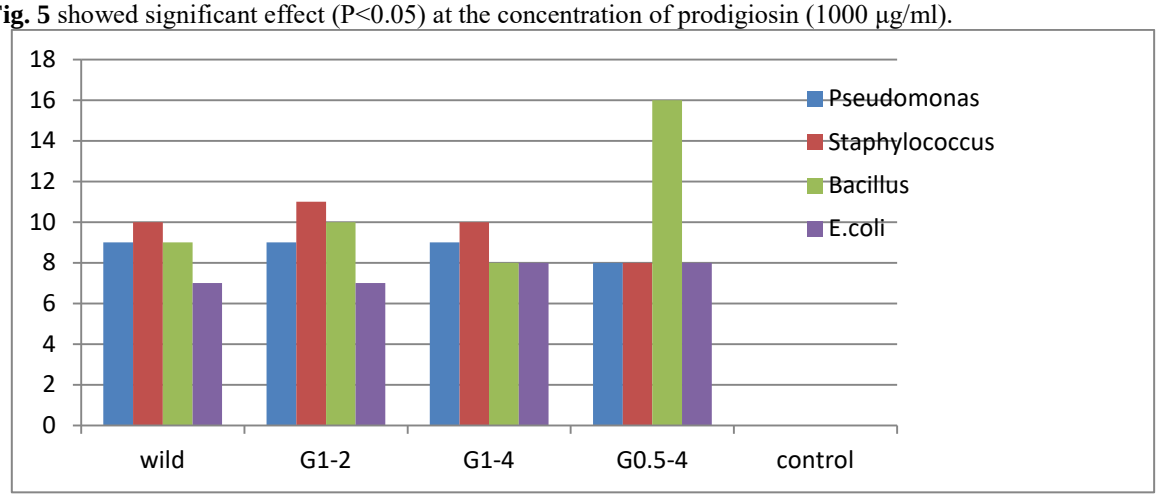

Fig 5. Effect of prodigiosin pigment of wild and other mutants' isolates of *S. marcescens* on growth of different bacteria, *Pseudomonas*, *Staphylococcus*, *Bacillus* and *E. coli*.

IV. Conclusion

The red pigment (prodigiosin) has an essential role in different domains: biological, malignant cell therapy, commercial, etc. Prodigiosin that was extracted from *S. marcescens* very easily to reduce the time and exertion when dialing with different domains above. After doing spontaneous mutation, betterments on amount and effect (quantity and quality).

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